

This listing of claims will replace all prior versions, and listings, of claims in the application.

**Listing of Claims:**

1. (Previously presented) A process for purifying correctly folded monomeric insulin-like growth factor-I (IGF-I) from a medium containing IGF-I peptides, comprising the steps of:

(a) contacting the medium with a sufficient quantity of a first cation exchange matrix under conditions allowing adsorption of at least about 95% of total IGF-I from the medium;

(b) washing the IGF-I-loaded first cation exchange matrix with a first cation exchange wash buffer, which removes adsorbed non-IGF-I material from said matrix and allows retention of authentic or non-authentic IGF-I by said matrix;

(c) eluting all forms of adsorbed IGF-I from the cation exchange matrix of step (a) by contacting said cation exchange matrix with a sufficient quantity of a first cation exchange elution buffer, which has a sufficiently high pH or ionic strength to displace substantially all of said authentic and non-authentic IGF-I from said cation exchange matrix;

(d) transferring the IGF-I-containing eluate from step (c) into an unfolding/refolding buffer, which:

(i) reduces the intrachain disulfide bonds of IGF-I protein and promotes unfolding without permanent denaturation; and

(ii) permits refolding of the IGF-I and reoxidation to form properly-paired intrachain disulfide bonds;

(e) contacting the properly folded IGF-I from step (d), after transfer into a suitable solvent system, with a sufficient quantity of a hydrophobic interaction chromatography matrix under conditions allowing adsorption of at least about 95% of said IGF-I from said eluate;

(f) washing the IGF-I-loaded hydrophobic interaction chromatography matrix with a hydrophobic interaction wash buffer having an ionic strength sufficiently low to remove most of the non-authentic IGF-I from the hydrophobic interaction chromatography matrix while retaining substantially all of the adsorbed authentic IGF-I on said matrix;

(g) eluting the adsorbed IGF-I from said hydrophobic interaction chromatography matrix by contacting said matrix with a hydrophobic interaction elution buffer, which has a

sufficiently elevated pH, or sufficiently low ionic strength, to cause displacement of substantially all of the adsorbed authentic IGF-I from said matrix;

(h) contacting the eluate from step (g) with a sufficient quantity of a second cation exchange matrix under conditions allowing adsorption of at least about 95% of the IGF-I from the eluate;

(i) washing the IGF-I-loaded second cation exchange matrix with a cation exchange wash buffer having a sufficiently high ionic strength, or sufficiently high pH, to remove non-authentic IGF-I from said matrix while retaining substantially all of the adsorbed authentic IGF-I on said matrix;

(j) eluting the adsorbed IGF-I from said second cation exchange matrix by contacting said matrix with a second cation exchange elution buffer, which has sufficiently high ionic strength, or sufficiently high pH, to displace substantially all of the adsorbed authentic IGF-I from said matrix;

(k) contacting the eluate from step (j), in an aqueous buffer, with a suitable quantity of a reverse phase chromatography matrix under conditions allowing adsorption of at least about 95% of the IGF-I from the eluate;

(l) washing the IGF-I-loaded reverse phase chromatography matrix with an aqueous/organic reverse phase wash buffer having an organic solvent concentration sufficiently high to remove non-authentic IGF-I from said matrix while retaining substantially all of the adsorbed authentic IGF-I on said matrix; and

(m) eluting the adsorbed IGF-I from said reverse phase chromatography matrix with an aqueous/organic buffer having an organic solvent concentration high enough to remove substantially all of the authentic IGF-I without removing substantially all of the multimeric forms of IGF-I from said matrix.

2. (Original) The process of claim 1, wherein the non-authentic IGF-I removed by the washing in step (f) is reprocessed at least once through steps (d) to (g), inclusive, prior to initiation of step (h).

3. (Original) The process of claim 1, wherein said IGF-I is human recombinant IGF-I.

4. (Original) The process of claim 3, wherein said human recombinant IGF-I is produced in transformed yeast cells.
5. (Original) The process of claim 4, wherein said transformed yeast cells are of the species *Pichia pastoris*.
6. (Original) The process of claim 5, wherein said species *Pichia pastoris* is of the strain GS115.
7. (Original) The process of claim 4, wherein said IGF-I is secreted into the yeast growth medium.
8. (Original) The process of claim 1, part (a), wherein said first cation exchange matrix is sulfolpropylated and in a chromatography column.
9. (Original) The process of claim 1, part (b), wherein said washing consists essentially of applying to said first cation exchange matrix approximately 3.0 column volumes of 0.02 M acetic acid, followed by approximately 3.0 column volumes of 0.05 M sodium acetate (pH 5.5).
10. (Original) The process of claim 1, part (c), wherein said eluting consists essentially of applying to said first cation exchange matrix between 3 and 10 column volumes of 0.05 M sodium acetate (pH 5.5) and 0.4 M sodium chloride.
11. (Original) The process of claim 1, part (d) wherein said unfolding/refolding buffer consists essentially of: between about 1.5 and 3.0 M urea; between about 1.0 and 3.0 M sodium chloride; between about 5% and 20% (v/v) ethanol; between about 1 mM and 15 mM sodium borate; and between about 0.005 mM and 10.0 mM dithiothreitol; and has a pH between about 8.5 and 10.0.

12. (Original) The process of claim 1, part (d) wherein said unfolding/refolding buffer consists essentially of 2 M urea, 1.5 mM sodium chloride, 15% ethanol, 5mM sodium borate and 0.2 mM DTT and has a pH between about 9.0 and 9.5.

13. (Original) The process of claim 1, part (e), wherein said hydrophobic interaction chromatography matrix is a butyl-substituted, polymethacrylate matrix.

14. (Original) The process of claim 1, part (f), wherein said washing consists essentially of applying to said hydrophobic interaction chromatography matrix approximately three column volumes of 0.2 M acetic acid, containing 0.5 M sodium chloride; followed by approximately ten column volumes of 0.2 M acetic acid, containing 0.15 to 0.25 M sodium chloride.

15. (Original) The process of claim 1, part (g), wherein said eluting consists essentially of applying to said hydrophobic interaction chromatography matrix approximately four column volumes of 0.2 M acetic acid, containing 0 to 0.02 M sodium chloride, and having a pH of approximately 3.0.

16. (Original) The process of claim 1, part (h), wherein said second cation exchange matrix is sulfylpropylated and in a chromatography column.

17. (Original) The process of claim 1, part (i), wherein said washing consists essentially of applying to said second cation exchange matrix approximately seven to ten column volumes of 0.05 M sodium acetate buffer, containing 0.1 M NaCl, and having a pH of approximately 5.5.

18. (Original) The process of claim 1, part (j), wherein said second cation exchange elution buffer consists essentially of approximately seven column volumes of 0.05 M sodium acetate, 0.4 M sodium chloride, having a pH of approximately 5.5.

19. (Original) The process of claim 1, part (k), wherein said reverse phase chromatography matrix is a polymer resin medium.

20. (Original) The process of claim 1, part (l), wherein said washing consists essentially of applying approximately one column volume of 0.2 M acetic acid, containing 0% ethanol, followed by approximately 4 column volumes of 0.2 M acetic acid, containing 19% ethanol.

21. (Original) The process of claim 1, part (m), wherein said eluting consists essentially of applying to said reverse phase chromatography matrix approximately four column volumes of 0.2 M acetic acid, containing 19% ethanol, followed by a gradient of 19% to 25% ethanol (v/v), in 0.2 M acetic acid, wherein the gradient is selected from the group consisting of linear gradient and step gradient.

22-26. (canceled)